

10/516779

DT12 Rec'd PCT/PTO 03 DEC 2004

ART 34 AMDT**WE CLAIM:**

- 1) A method of modifying a target nucleic acid of interest at a target locus within a genome of a host comprising:
 - a) introducing into the host a gene targeting construct (GTC) and culturing the host so as to:
 - i) express the gene targeting construct encoding a gene targeting RNA, to produce the gene targeting message RNA capable of self-priming reverse transcription by a reverse transcriptase (RT);
 - ii) reverse transcribe at least a portion of the gene targeting message RNA to produce an *in vivo* gene targeting substrate (GTS) having a gene targeting nucleotide sequence (GTNS), wherein the GTNS is homologous to the target locus and comprises a sequence modification compared to the target nucleic acid; and,
 - b) selecting a host having the sequence modification at the target locus.

- 2) The method of claim 1, wherein the host is capable of expressing the RT prior to transforming the host with the gene targeting construct.

- 3) The method of claim 1, wherein the host is modified to be capable of expressing the RT at the same time as, or after, transforming the host with the gene targeting construct.

- 4) The method of claim 1, 2 or 3, wherein the GTC is introduced into the host by transformation, by cross breeding or by cell fusion.

- 5) A gene targeting construct comprised of recombinant nucleic acid sequences in a host having a host genome, wherein the gene targeting construct encodes and is capable of expressing a gene targeting message RNA, wherein the gene targeting message RNA is capable of self-priming reverse transcription by a reverse transcriptase in the host to produce a gene targeting substrate having a gene targeting nucleotide sequence,

wherein the gene targeting nucleotide sequence is homologous to a target locus in the host genome and comprises a sequence modification compared to the target locus, wherein expression of the gene targeting construct in the host introduces the sequence modification as a heritable genetic change
5 in the target sequence in the genome of the host .

- 6) The gene targeting construct of claim 5, wherein the gene targeting construct comprises an msr coding region and an msd coding region.
- 10 7) A recombinant reverse transcriptase coding sequence encoding a reverse transcriptase having a nuclear localization signal sequence.
- 15 8) The gene targeting construct of claim 6, wherein the msr and msd coding regions are in operative association with a first regulatory region, and the construct further comprises a nucleotide sequence encoding a reverse transcriptase.
- 20 9) The gene targeting construct of claim 8, wherein the nucleotide sequence encoding the reverse transcriptase is in operative association with the first regulatory region or with a second regulatory region.
- 10) The gene targeting construct of claim 8, wherein the reverse transcriptase comprises a nuclear localization signal sequence.
- 25 11) The gene targeting construct of claim 8 wherein the regulatory region is operatively active in an S phase, a G1/S boundary of a cell cycle, or during meiosis.
- 30 12) The gene targeting construct of claim 11, wherein the regulatory region is selected from the group consisting of a histone promoter, a cyclin promoter, a promoter associated with a gene involved in DNA replication, a promoter associated with a gene involved in DNA repair and a promoter associated with a gene involved in DNA homologous recombination.

- 13) The gene targeting construct of claim 8, further comprising a nucleotide sequence encoding a selectable marker.
- 14) A vector comprising the gene targeting construct of any one of claims 8 through 13.
5
- 15) An host comprising the vector of claim 14.
- 16) The host of claim 15, selected from the group consisting of a plant cell, an animal cell, a yeast cell, and an insect cell.
10
- 17) The host of claim 16, wherein the host is a plant cell.
- 18) A method of modifying a target locus in a host comprising transforming the host with the gene targeting construct of any one of claims 8 through 13.
15
- 19) A method of modifying a target locus in a host comprising transforming the host with the vector of claim 14.
20
- 20) The method of claim 19 wherein the host is a eukaryotic organism.
- 21) The method of claim 20, wherein the host is selected from the group consisting of a plant cell, an animal cell, a yeast cell, and an insect cell.
25
- 22) The gene targeting construct of any one of claims 1 through 6, wherein the gene targeting nucleotide sequence comprises one, or more than one, region of 15 to about 500 nucleotides, exhibiting about 70% to about 99% sequence similarity with the target locus sequence, as determined using the following conditions: Program: blastp; Database: nr; Expect 10; filter: default; Alignment: pairwise; Query genetic Codes: Standard (1).
30
- 23) The gene targeting construct of claim 22, wherein the one or more than one region is of less than 300 nucleotides in length.